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Immunological Abnormalities in Splenic Marginal Zone Cell Lymphoma

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The clinical features of patients with splenic marginal zone cell lymphoma (SMZCL) have rarely been reported. In the present study, immunological abnormalities, particularly hematological abnormalities, observed in SMZCL were described. Autoimmune hemolytic anemia, immune thrombocytopenia, and appearance of lupus anticoagulant were observed in 2 of 3 patients with SMZCL. Other abnormal data including monoclonal gammopathy and cold agglutinin were also observed in 2 of the 3 patients. Immunological abnormalities may be characteristic complications in patients with SMZCL and must be followed carefully, since they may be a reliable marker of this type of lymphoma activity. Am. J. Hematol. 56:173–178, 1997. © 1997 Wiley-Liss, Inc.

Key words: splenic lymphoma; splenic marginal zone; autoimmune hemolytic anemia; thrombocytopenia; lupus anticoagulant; anticardiolipin antibody; cold agglutinin

INTRODUCTION

Although splenic involvement is frequently observed in patients with malignant lymphoma, especially in those with disseminated lymphoma, primary splenic lymphomas have rarely been diagnosed. However, in recent years, several investigators have reported such cases and have proposed criteria for this entity. Primary splenic lymphoma has been defined, to date, as being the involvement of the spleen and splenic hilar lymph nodes without the involvement of other organs [1–3]. This disorder is a non-Hodgkin's lymphoma and B-cell phenotype in most cases [1,2]. However, primary splenic lymphomas rarely cause symptoms until they have disseminated, and the above criteria have recently been modified. The criteria for this disorder are now expanded to include cases in which the greatest tumor is found in the spleen with the rare involvement of other organs (e.g., liver and bone marrow) [4–6]. In the spleen, the follicular mantle is separated into two zones surrounding the germinal center. The inner side is termed the mantle zone and the outer side, the marginal zone. Marginal zone cells have mainly B-cell phenotype and medium-size lymphocytes [6,7]. Schmid et al. [6] reported 4 cases of splenic marginal zone cell lymphoma (SMZCL) and described the pathological and histochemical characteristics.

In the present paper, we describe three cases with SMZCL and characterize the clinical features, especially the immunological abnormalities.

CASE REPORT

Case 1

A 57-year-old woman was admitted to Gunma University Hospital with purpura of the lower extremities in March 1990. Physical examination revealed anemia and splenomegaly without lymphadenopathy, as well as the purpura of lower extremities. Pertinent peripheral blood laboratory data were: Hb 7.8 g/dl, platelet count 98 × 10^9/l, and white blood cell (WBC) count 4.7 × 10^9/l with atypical lymphocytes with pale cytoplasm. In the bone marrow, atypical lymphocytes accounted for 3.6% of all

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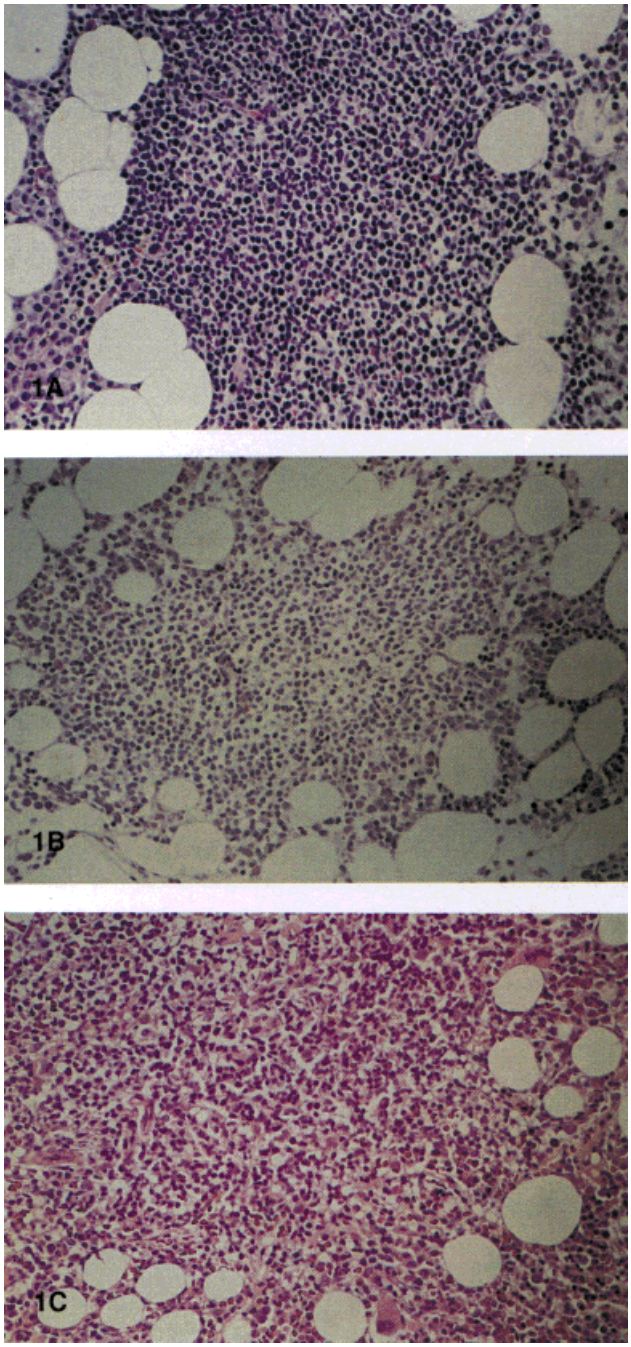

Fig. 1. Clot section of bone marrow of case 1 (A), case 2 (B), and case 3 (C). Focal proliferations of lymphoma cells are observed.

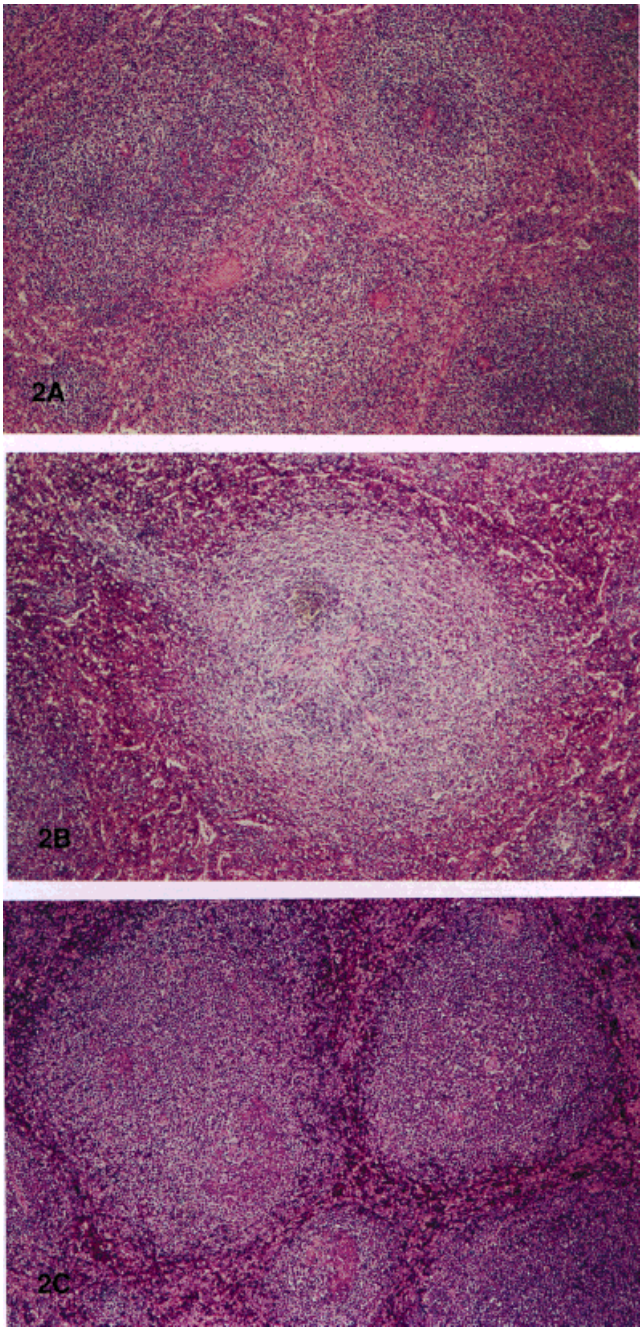

Fig. 2. Histological appearance of spleen of case 1 (A), case 2 (B), and case 3 (C). In each case, atypical cells infiltrate to and enlarge the perifollicular zone.

nucleated cells (Fig. 1A). The serum lactate dehydrogenase (LDH), copper, and creatinine values were 333 IU/l, 173 μg/dl, and 1.9 mg/dl, respectively. The results of direct and indirect Coombs test were negative. Platelet-associated IgG (PAIgG) was 92.5 ng/10^7 platelets. Cold agglutinin was positive. A computed tomography (CT) scan revealed splenomegaly without lymphadenopathy. On the 44th hospital day, splenectomy and liver biopsy were performed, and a diagnosis was made of SMZCL associated with liver infiltration (Figs. 2A and 3A). The tumor cells in the spleen were CD3(−), CD5(−), CD45RO(−), CD20(+), PRAD1(−), and surface IgM, λ(+). Chromosomal analysis revealed normal karyotype. The patient was discharged and treated as an outpatient receiving 10 courses of COP therapy (cyclophosphamide, vincristine, and prednisolone). In October 1992, she complained of general fatigue and an increase in

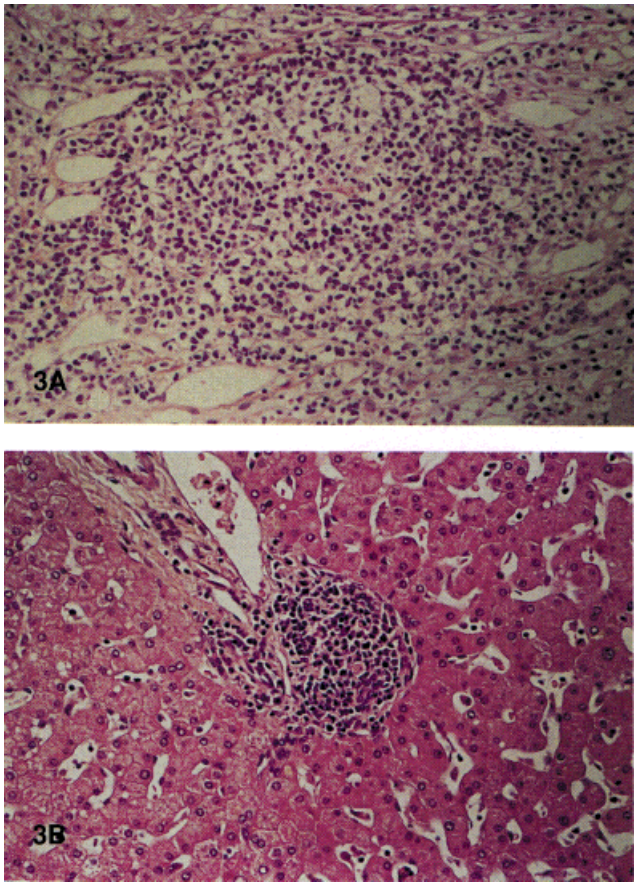

Fig. 3. Histological appearance of liver of case 1 (A) and case 3 (B). Nodular infiltration of lymphoma cells is observed.

weight. At this time, the values for TSH, free T3, free T4, antimicrosome antibody, and antithyroglobulin antibody were 6.4 μU/l, 1.7 pg/dl, 0.3 ng/dl, 1:1,600, and 1:1,600, respectively. She was diagnosed as having hypothroidism, and treated with L-thyroxine. In March 1995, lymphoma cell infiltration in bone marrow was still observed. She was treated with 6 courses of COP therapy. PAIgG, cold agglutinin, antimicrosome antibody, and antithyroglobulin antibody were improved but still positive. She is without symptomes at 81 months of follow-up.

Case 2

A 59-year-old woman was admitted to Gunma University Hospital with abdominal distension in February 1991. Physical examination showed anemia and hepatosplenomegaly without lymphadenopathy. Pertinent peripheral blood data were: Hb 10.7 g/dl, platelet count 67 $\times 10^9$/l, and WBC count 5.0 $\times 10^9$/l. In the bone marrow, erythroblastosis and the infiltration of atypical lymphocytes were observed (Fig. 1B). Serum GOT, GPT, LDH, direct bilirubin, indirect bilirubin, and haptoglobin values were 241 IU/l, 12 IU/l, 741 IU/l, 3.1 mg/dl, 2.6 mg/dl, and 16.1 mg/dl, respectively. The result of the direct Coombs test was positive and PAIgG was 307.9 ng/10^7 platelets. She was diagnosed as having Evans syndrome and treated with prednisolone (1 mg/kg) given orally. After treatment, the patient's hemolytic anemia, thrombocytopenia, and splenomegaly improved; however, the WBC and mature lymphocyte counts increased to 14.4 $\times 10^9$/l and 9.2 $\times 10^9$/l, respectively. Surface μ, κ, CD20, and HLA-DR were positive and rearrangement of the JH and Jκ genes was detected. CD5 was negative. With the reduction of the prednisolone dose, the lymphocyte count decreased and the spleen returned to its original size. The patient was subsequently maintained with prednisolone (10 mg/body/day) In October 1992, she was re-admitted with general fatigue and recurrence of the thrombocytopenia. Values for peripheral blood were: Hb 12.4g/dl, platelet count 64 $\times 10^9$/l, and WBC count 4.1 $\times 10^9$/l. Serum LDH and haptoglobin were 571 IU/l, and 10.9 ng/dl, respectively. The result of the direct Coombs test was positive and PAIgG was 128.9 ng/10^7 platelets. At this time, lupus anticoagulant, determined by the method of Rosove et al. [8], was positive, but anti-cardiolipin antibody, determined by the solid phase enzyme immunoassay of Koike et al. [9], was negative. Serum protein concentration and electrophoretic profiles were normal; however, monoclonal gammopathy (IgG, κ) was observed. A splenectomy was performed and SMZCL was diagnosed (Fig. 2B). Surface marker analysis was not performed. The spleen cells showed normal karyotype and rearrangement of the JH and J κ genes. In March 1993, the platelet count had returned to normal, but the level of PAIgG was still high. The results of the direct Coombs test, tests for lupus anticoagulant, and monoclonal gammopathy were negative. In January 1996, the patient was re-admitted to our hospital with severe lumbago. X-ray, CT scan, and magnetic response imaging (MRI) of the spine revealed lytic lesion of L2 and L3. She was diagnosed as having lumbar spine metastasis of lymphoma, and received Linac irradiation of the lumbar spine and a course of CHOP therapy 6 times. She recovered without further therapy, and her PAIgG became negative.

Case 3

A 58-year-old woman was admitted to our hospital with general fatigue in September 1991. Physical examination showed anemia and hepatosplenomegaly without lymphadenopathy. Pertinent peripheral blood data were: Hb 7.9 g/dl, platelet count 155 $\times 10^9$/l, and WBC count 1.6 $\times 10^9$/l, with a few atypical cells. In the bone marrow, erythroblastosis was observed and the percentage of atypical cells was 2.0%. On clot section, focal proliferation of B cells was observed (Fig. 1C). Values for serum GOT, GPT, LDH, direct bilirubin, indirect bilirubin, and haptoglobin were 18 IU/l, 8 IU/l, 849 IU/l, 1.2 mg/dl, 0.9

TABLE I. Clinical Features of Splenic Marginal Zone Cell Lymphoma Patients*

	Our patients	Schmid et al. [6]	Pawade et al. [10]	Rosso et al. [12]	Dierlamm et al. [13]	Wu et al. [14]	Hammer et al. [15]	Total
No. of patients	3	4	14	3	10	5	14	53
Male/female	0/3	0/4	6/8	1/3	2/8	2/3	5/9	16/37
Age	57,58,59	43,61,67,72	60–81	67,69,78	44–74	62–73	35–79	35–81
Median age	—	—	64.5	—	57	68.4	68	65
Extrasplenic involvement								
Bone marrow	3/3	4/4	8/14	1/3	10/10	5/5	8/11	39/50 (78%)
Liver	2/3	1/4	N.C.	1/3	N.C.	4/5	5/7	13/22 (59%)
Peripheral lymph nodes	0/3	0/4	N.C.	0/3	3/10	2/5	2/14	7/39 (18%)
Splenic hilar lymph nodes	0/3	3/4	5/14	0/3	N.C.	2/2	7/14	17/40 (43%)
Prognosis	Good	Good	Good	Good	Good	Good	Good	Good

*N.C., not cited.

mg/dl, and 5.1 mg/dl, respectively. Serum protein concentration and electrophoretic profiles were normal; however, immunoelectrophoresis revealed IgM λ monoclonal gammopathy. The result of the direct Coombs test was positive but PAIgG was negative. Cold agglutinin was positive. The prothrombin time was normal, but the activated partial thromboplastin time was prolonged. Lupus anticoagulant was determined by the method of Rosove et al. [8] and there was a false-positive result on the serologic test for syphilis. Results of the test for anticardiolipin antibody, performed by the solid phase enzyme immunoassay of Koike et al. [9], were positive for IgM but negative for IgG. Splenectomy was performed on the 27th hospital day, and SMZCL was diagnosed (Fig. 2C), accompanied by autoimmune hemolytic anemia (AIHA) and lymphoma cell infiltration of the liver (Fig. 3B). The tumor cells in the spleen were CD5(−), CD20(+), CD21(+), PRAD 1(−), and surface IgM, λ(+). The karyotype of lymphoma cells was normal. DNA analysis revealed JH rearrangement. After splenectomy, the signs of hemolysis disappeared. She was then treated with MCNU-CHOP therapy (ranimustine, cyclophosphamide, doxorubicin, vincristine, prednisolone). She was discharged and 6 courses of chemotherapy (pirarubicin, cyclophosphamide, vincristine, prednisolone) were administered. In October 1992, atypical cells were not present in the bone marrow or peripheral blood. The result of the Coombs test and cold agglutinin was negative. The lupus anticoagulant, anticardiolipin antibody, and false-positive serologic test for syphilis were no longer positive, and M-protein disappeared. Chemotherapy was discontinued at this time. She has remained asymptomatic at 68 months of follow-up.

DISCUSSION

The marginal zone is a characteristic structure in the spleen of mammals. The follicular mantle of the human spleen consists of two layers: the mantle zone on the inner layer and the marginal zone on the outer layer. The marginal zone lymphocytes have a specific enzyme- and immunophenotype, which differ from that of the mantle zone lymphocytes [6,7]. The marginal zone lymphocytes are positive for alkaline phosphatase but negative for IgD and KiB3. Most mantle cell lymphomas express CD5 and have the t(11;14)(q13:q32) translocation [10]. In addition, immunohistochemical expression of PRAD 1/cyclin D1 protein was reported in the tumor cells in mantle cell lymphoma [11]. Schmid et al. [6] reported four cases with splenic lymphoma arising from the marginal zone and named the entity ''splenic marginal zone cell lymphoma.'' The histology of the spleen was characterized by broad concentric strands of monomorphic medium-sized lymphocytes with round or indented nuclei and moderate amounts of pale cytoplasm. These cells surrounded follicle centers. Clinically, all four of the patients were women past middle age who presented with splenomegaly and anemia, and in whom there was bone marrow involvement. All except one patient had a good prognosis after splenectomy without further chemotherapy.

We encountered three cases compatible with the pathologic criteria of Schmid et al. [6]. The lymphoma cells of our 3 patients were negative for CD5. PRAD 1/cyclin D1 protein was not detected in our 2 examined cases. Clinically, our patients were also women past middle age (57, 58, and 59 years) and had marked splenomegaly without lymphadenopathy. However, tumor cell infiltration of bone marrow and liver was observed. Tumor cells were also found in peripheral blood. The 3 patients of the present study remain well at 76, 41, and 59 months, respectively, after splenectomy. The clinical features of SMZCL of our and previously reported patients are summarized in Table I [6,10,12–15]. The number of females is twice the number of males, and most patients were past middle age. Marked splenomegaly, bone marrow and liver infiltration of lymphoma cells were frequently observed; however, peripheral lymph node involvement is rare. SMZCL has a good prognosis after splenectomy.

TABLE II. Immunological Abnormalities in Splenic Marginal Zone Cell Lymphoma Patients*

	Our cases			Schmid et al. [6]	Pawade et al. [10]	Rosso et al. [12]	Dierlamm et al. [13]	Wu et al. [14]	Hammer et al. [15]	Total (53 cases)
	Case 1	Case 2	Case 3	(4 cases)	(4 cases)	(3 cases)	(10 cases)	(5 cases)	(14 cases)	(%)
Coombs test	−	+	+	N.C.	2/14	1/3	2/10	N.C.	N.C.	7/30 (23)
PAIgG	+	+	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	2/3 (67)
Lupus anticoagulant	−	+	+	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	2/3 (67)
Anticardiolipin antibody	−	−	+	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	1/3 (33)
Monoclonal gammopathy	−	+	+	N.C.	3/14	N.C.	6/10	1/5	N.C.	12/32 (38)
Thyroid test	+	−	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	1/3 (33)
Microsome test	+	−	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	1/3 (33)
Cold agglutinin	+	−	+	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	2/3 (67)
Anti-nuclear factor	−	−	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	0/3 (0)
Anti-DNA antibody	−	−	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	0/3 (0)
Rheumatoid factor	−	−	−	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	0/3 (0)

*N.C., not cited.

Despite the bone marrow infiltration, the anemia in these patients appeared to be due to an autoimmune mechanism. Apart from the AIHA, the immunological abnormalities observed in the SMZCL were almost all hematological abnormalities (Table II). It has been reported that in 14% of patients with AIHA, the anemia was caused by lymphoproliferative disorders, and that in almost 2% of patients with non-Hodgkin's lymphoma, there was associated AIHA [16–18]. AIHA was observed in 2 of our 3 patients (67%) and 7 of the 30 (23%) reported patients with SMZCL. This incidence was higher than that reported in other lymphomas.

Immune thrombocytopenia (ITP) has been reported to be associated with non-Hodgkin's lymphoma and Hodgkin's disease at rates of 0.4 and 1.5%, respectively [16,19]. This complication was observed in 2 of our 3 patients (67%); however, no SMZCL patient with this complication has been reported in the literature.

In addition, lupus anticoagulant was detected in two of our patients and cardiolipin antibody in one. Lupus anticoagulant, which induces a prolongation of phospholipid-dependent clotting times such as prothrombin time and partial thromboplastin time, has been found in patients with systemic lupus erythematosus (SLE) [8,9]. Ciaudo et al. [20] reported four cases of primary lymphoplasmacytic lymphoma of the spleen associated with lupus anticoagulant and monoclonal IgM. Our patients were similar to theirs, but ours had additional immunological abnormalities and did not have SLE-like abnormalities (antinuclear antibody, anti-DNA antibody). Despite the remarkable abnormalities on the coagulation tests, none of our patients presented any thrombotic or hemorrhagic complications, even when they underwent splenectomy.

Interestingly, none of our patients had associated rheumatoid diseases, such as Sjogren's syndrome, rheumatoid arthritis, SLE, or scleroderma, which are reported to be frequent complications of malignant lymphoma [21,22].

It is unclear why immunological abnormalities are associated with SMZCL. Splenic marginal zone cells are considered to represent a noncirculating population of memory cells that migrate to the germinal center of follicles in response to immunological stimuli [23–26], and the neoplastic transformation of these cells may disturb the immunomechanism and induce immunological abnormalities.

In case 1, lymphoma cells remained in the bone marrow despite the splenectomy and chemotherapy, and all autoantibodies observed before the splenectomy remained positive. Similarly, in case 2, morphological and DNA analyses showed lymphoma cells in bone marrow and peripheral blood after splenectomy. However, all autoantibodies except for PAIgG were negative at this time. In case 3, in contrast, the lymphoma cells and autoantibodies were eliminated by splenectomy and chemotherapy. Therefore, we suspected that these immunological abnormalities were caused by SMZCL. These immunological abnormalities must be followed carefully, since they may be reliable markers of this type of lymphoma activity.

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